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COMMUNICATION

Functionalized graphene oxide as nanocatalyst in Functionalized graphene oxide as nanocatalyst
dephosphorylation reaction: pursuing artificial **enzymes enzymes**

Cite this: DOI: 10.1039/x0xx00000x Cite

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Received 00th January 2012, Accepted 00th January 2012 Received 00th
Accepted 00th
DOI: 10.1039/x

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www.rsc.org/ www.rsc.org/

The present study reports for the first time the use of thiol-The present study reports for the first time the use of thiol-
functionalized graphene oxide nanocatalyst with impressive activity (>10⁵-fold) in dephosphorylation reactions. The **innovative and recyclable nanocatalyst has potential in designing artificial enzymes with targeted multifunctionalities and in detoxification of organophosphorus agents.** innovative and recyclable nanocatalyst
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and in detoxification of organophosphorus ized graphene oxide nanocatalyst with impressive >10⁵-fold) in dephosphorylation reactions. The and recyclable nanocatalyst has potential in artificial enzymes with targeted multifunctionalities

Dephosphorylation reactions are vital in biological systems,
such as in regulatory and signaling processes, which are mediated by extraordinarily efficient enzymes. Particularly, reversible phosphorylation of protein dictates many cell life functionalities. Herein, the thiol group, mainly involved in oxidative thiol oxidative thiol-disulfide pathways, rarely rarely occurring cysteine cysteine-based phosphatases, *e.g.*, bacterial antibiotic resistance.² Moreover, many aspects regarding mechanistic elucidation and detection of intermediates in protein phosphorylation reactions are still not clear.³ Therefore, mimicking enzymatic active sites is strategic for designing novel artificial enzymes and potential enzymatic inhibitors for therapeutic purposes. Dephosphorylation reactions are vital in biological systems, such as in regulatory and signaling processes, which
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multifunctional and versatile, hence, the complex enzymatic architecture can be more readily mimicked, different functionalities on the backbone of these materials. The two dimensional sheets with sp^2 carbons of graphene comprise many optimum chemical, mechanical and electrical properties,⁴ many optimum chemical, mechanical and electrical properties,⁴ thus prompting promising applications in nanoelectronics, biomedicine, sensors and artificial enzymes. graphene precursor is graphene oxide (GO), which is obtained multifunctional and versatile, hence, the complex enzym architecture can be more readily mimicked, *e.g.*, by ancho different functionalities on the backbone of these materials. thus prompting promising applications in nanoelectronics,
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Functionalized graphene oxide as nanocatalyst in dephosion from the set of th followed by chemical reduction, yielding graphene (or the socalled reduced-graphene oxide, rGO). Hence, targeted covalent functionalization of the oxygenated groups on GO can enhance functionalization of the oxygenated groups on GO can enhance followed by chemical reduction, yielding graphene (or the so-
called reduced-graphene oxide, **r**GO). Hence, targeted covalent
functionalization of the oxygenated groups on GO can enhance
the materials properties and extend Recently, we reported the thiolation of GO, by anchoring cysteamine (CA) by amide bonds on the carboxylate sites, functionalization of the oxygenated groups on GO can enhable materials properties and extend further application Recently, we reported the thiolation of GO, by ancho cysteamine (CA) by amide bonds on the carboxylate s unde thoroughly controllable features in nanocomposites with silver nanoparticles. Insofar, the freely available thiol groups on GOSH can efficiently act as nucleophiles in many reactions, although GOSH cannot be dispersed in any solvent, especially in water. In this context, we report GOSH as nanocatalyst in heterogeneous dephosphorylation reactions, using the activated in water. In this context, we report GOSH as nanocatalyst in heterogeneous dephosphorylation reactions, using the activated triester diethyl 2,4-dinitrophenyl phosphate (DEDNPP) as a model (Fig. 1).⁸ The proposed nanocatalyst can be readily separated and recycled consecutively, maintaining its catalytic activity. activity by oxidizing graphite, leading to a highly oxygenated surface,
followed by chemical reduction, yielding graphene (or the so-
called reduced-graphene oxide, rGO). Hence, targeted covalent thoroughly characterized and showed impressive size
controllable features in nanocomposites with silver
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Fig. 1. Reaction of DEDNPP with the nanocatalyst GOSH.

S Solid GOSH was added to a buffered solution at different pH values pH values, kept under controlled temperature (20°C) and Solid GOSH was added to a buffered solution at different pH values, kept under controlled temperature (20°C) and constant magnetic stirring and the reaction initiated by adding Solid GOSH was added to a buffered solution at different pH values, kept under controlled temperature $(20^{\circ}C)$ and constant magnetic stirring and the reaction initiated by adding an aliquot of DEDNPP. Periodically, sti

the solid was decanted and the remaining solution was the solid was decanted and the remaining solution was monitored by UV-Vis spectroscopy, following the appearance of the product 2,4 2,4-dinitrophenolate (DNP) at 400 nm. Rate of the product 2,4-dinitrophenolate (DNP) at 400 nm. Rate constants (k_{obs}) were obtained from the pseudo-first order kinetic profiles and correlated with the mass of catalyst used and the local concentration of reactive thiol groups on the GO surface, obtained by thermogravimetric data (TGA, Supplementary Information reproducible pseudo first-order profiles are consistent with heterogeneous catalytic reactions where kinetic control overcomes diffusion contributions. 9, characterization were carried out using TGA (under Ar) infrared (FTIR) and Raman spectroscopy. Full description of the characterization techniques are given in the Supplementary Information. Table 1 presents data obtained, along with comparatives to assign the catalytic efficiency of the reaction proposed. efficiency of the reaction proposed. kinetic profiles and correlated with the mass of catalyst
and the local concentration of reactive thiol groups on the
surface, obtained by thermogravimetric data (T
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Table 1 – Kinetic parameters obtained for reactions of DEDNPP with GOSH

^a Second order rate constant: ratio of the pseudo-first order rate constant and the concentration of thiol groups 8.42×10^{-4} mol L⁻¹/mg of solid GOSH, calculated by TGA analysis; calculated by TGA analysis; b Considering the total mass of catalyst used; Comparative using the second order rate constant $(M^{-1}s^{-1})$ for the reaction of DEDNPP with GOSH, H_2O (spontaneous reaction)⁸ and CA. ^a Second order rate constant
the concentration of thiol
calculated by TGA analysis
^c Comparative using the sec
DEDNPP with GOSH, H_2O **b** Considering the total mass of catalyst used;

 $1s^{-1}$), which increase with higher pH. Indeed, the alkaline hydrolysis of phosphate esters reaction at higher pH, although results show that solely GOSH acts proficiently, regardless of the parallel reaction with OH observed by the high rate constants at pH 8 (k _{GOSH}). Moreover, mild reaction condition (e.g. lower pH) is desirable when promoting greener catalytic reactions, as performed by our proficient biological enzymes. It should be noted that the reaction conversion is 100%, although no selectivity can be accounted, since only one substrate was evaluated infer catalytic activity, rate constants in the presence of GOSH were compared to the analogous spontaneous hydrolysis reaction (k_{H2O}) of DEDNPP, giving rate enhancements $(k_{\text{GOSH}}/k_{\text{H2O}})$ over 10⁵-fold, which are among the highest increments reported for heterogeneous dephosphorylation increments reported for heterogeneous dephosphorylation reactions.^{12, 13} Comparing the proposed heterogeneous catalysis with an analogous homogenous reaction, DEDNPP was reacted solely with CA, Surprisingly, rate constants in the presence of the nanocatalyst GOSH are 14-fold higher than with CA (k_{GOSH}/k_{CA}). Normally, a homogenous reaction is more efficient than its analogous heterogeneous reaction, catalytic efficiency of the proposed nanocatalyst. Also, one would expect CA (with same concentration of thiol groups on GOSH) to promote similar enhancements. These results indicate synergistic effects on the multifunctional nanocatalyst. Results show impressive second order constants (k _{GOSH}, M⁻ hydrolysis of phosphate esters¹¹ contributes to the overall reaction at higher pH, although results show that solely GOSH acts proficiently, regardless of the parallel reaction with OH, as observed by the high rate cons with an analogous homogenous reaction, DEDNPP was reacted solely with CA, *i.e.*, the reactive thiol group present on GOSH. Surprisingly, rate constants in the presence of the nanocatalyst GOSH are 14-fold higher than wit a homogenous reaction,¹² thus c
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ved by the high rate constants at *i.e.*, the reactive thiol group present on GOSH. 12 thus corroborating the prominent ¹¹ contributes to the overall 10⁵-fold, which are amoved for heterogeneous deparing the proposed heterogeneous reaction, DEDI, the reactive thiol group pronous in the presence of higher than with CA (k_{GOSH}) It should be noted that the
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ments rep enous reaction is more efficient than its analogous eous reaction,¹² thus corroborating the prominent efficiency of the proposed nanocatalyst. Also, one pect CA (with same concentration of thiol groups on the solution was decayed and the concentration was decayed and the product 2.4-diam results of the product 2.4-diam enterpretate (ONP) at 400 nm. Rate kinetic profiles and concentration with the mass of catalyst used inte ich increase with higher pH. Indeed, the alkaline
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We propose that the additional effects contributing to the overall catalysis can be due to attractive interactions of DEDNPP by the nanocatalyst, but further studies are needed to corroborate this proposition corroborate this proposition. The substrate GO domains, common for carbon derived materials this process concentrates reactants, thus optimizing the catalysis, as in micellar catalysis.¹⁶ Comparing the rate constant GO domains, common for carbon derived materials^{14, 15} and this process concentrates reactants, thus optimizing the catalysis, as in micellar catalysis.¹⁶ Comparing the rate constant per mass of catalyst (k_{GOSH} , more impressive¹² due to the low mass of catalyst used (1) mg), ideally desired in heterogeneous reaction. mg), ideally desired heterogeneous reaction. We propose that the additional effects contributing to the overall catalysis can be due to attractive interaction.
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Regarding the mechanism of the reaction, we propose that DEDNPP can adsorb on the GOSH surface and react with its mg), ideally desired in heterogeneous reaction.
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functional groups, as shown in Fig. 2: the highly nucleophil thiolate active species can attack the phosphorus atom leading thiolate active species can attack the phosphorus atom leading
to an unstable intermediate (**Int.**, Fig. 2) and the leaving group DNP DNP. Regarding the mechanism of the reaction, we propose that DEDNPP can adsorb on the GOSH surface and react with its functional groups, as shown in Fig. 2: the highly nucleophilic thiolate active species can attack the phosph can attack the phosphorus atom leading %) with similar studies is even
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Fig. 2. Proposed mechanism for the reaction of GOSH with DEDNPP.

Potentiometric titration was also carried out to determine the pK_a value for the thiol group on GOSH (see Supplementary the pK_a value for the thiol group on GOSH (see Supplementary Information). Results indicate an unexpected low pK_a of Information). Results indicate an unexpected low pK_a of 6.46±0.02 for the thiol moiety of GOSH, in contrast to CA with pK_a of 8.35.¹⁷ Although, this lower pK_a is common in complex systems (proteins), 18 with neighboring groups that can stabilize systems (proteins),¹⁸ with neighboring groups that can stabilize the thiolate, such as the alcohol groups available on GO the thiolate, such as the alcohol groups available on GO surfaces. Hence, over the pH range studied, thiolate species are present predominantly present predominantly on GOSH, consistent with the proposed mechanism. As in similar nucleophilic reactions, 8 the putative phosphorylated surface of GOSH (hydrolyses, regenerating the thiol sites for successive reactions.
Another possible mechanism is the nucleophilic attack by
thiolate on the aromatic carbon (C-O cleavage). Nevertheless, Another possible mechanism is the nucleophilic attack by thiolate on the aromatic carbon (C-O cleavage). Nevertheless, this path should not contribute significantly to the overall reaction, since the absorbance variation profile shows early formation of DNP, consistent with attack on phosphorus atom.¹⁹ Indeed, on-going mechanistic studies involving the reaction of CA with DEDNPP by mass spectrometry confirms the presence of the phosphorylated intermediate formed via the this path should not contribute significantly to the overall
reaction, since the absorbance variation profile shows early
formation of DNP, consistent with attack on phosphorus
atom.¹⁹ Indeed, on-going mechanistic studie be noted that in the reaction conditions carried out, only the thiol groups of CA are reactive, since the nitrogen atom is reaction of CA with DEDNPP by mass spectrometry confirms
the presence of the phosphorylated intermediate formed via the
thiolate attack, that should analogously occur herein.²⁰ It should
be noted that in the reaction co nucleophile.¹⁷ Due to this higher pK_a of the potentially nucleophilic amine site, CA reactivity increases more upon increasing pH, compared to GOSH. Additionally, the formation nucleophilic amine site, CA reactivity increases more upon nucleophilic amine site, CA reactivity increases more upon increasing pH, compared to GOSH. Additionally, the formation surfaces. Hence, over the pH range studied, thiolate species are
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of disulphide bond, which would inhibit the reactivity of CA, was discarded since for GOSH, p previous characterizations indicate freely available thiol groups. formation does not seem to contribute significantly during the reaction time followed. of disulphide bond, which would inhibit the reactivity of CA,
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filtration and reused as catalyst in new reactions with DEDNPP (three cycles). Fig carried out, along with rate constants for each cycle evaluated filtration and reused as catalyst in new reactions
(three cycles). Fig. 3 illustrates with photos how
carried out, along with rate constants for each cyc infer recycling features, GOSH was recovered by
betweed as catalyst in new reactions with DEDNPP
Fig. 3 illustrates with photos how recycling was In order to infer recycling features, GOSH was recovered by

Results confirm that the highly efficient nanocatalyst GOSH maintains its catalytic property over several reaction cycles, suggesting its reactive groups maintain intact decrease in reactivity upon recycling can be attributed do adsorbed species such as the phosphate inorganic product. Although, even after the third cycle, GOSH is very effective: 2.6×10^5 -fold increment, compared to the spontaneous reaction, in contrast to 3×10^5 -fold observed in the first cycle. Overall, the nanocatalyst is easily recovered from reaction medium and can be recycled consecutively, heterogeneous reactions. Results confirm that the highly efficient nanocatalyst GOSH suggesting its reactive groups maintain intact. The slight decrease in reactivity upon recycling can be attributed do adsorbed species such as the phosphate inorganic product. Although, even after the third cycle, GOSH is nanocatalyst is easily recovered from reaction medium and can
be recycled consecutively, which is desired in catalytic
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overall properties and functionalities were confirm
various characterization techniques: TGA, FTIR and
spectroscopy. FTIR spectra for GOSH taken before an
reaction with DEDNPP are given in Fig. 4. various characterization techniques: TGA, FTIR and Raman spectroscopy. FTIR spectra for GOSH taken before and after spectroscopy. FTIR spectra for GOSH takent reaction with DEDNPP are given in Fig. 4. the reused GOSH, the preservation of its
and functionalities were confirmed by
ion techniques: TGA, FTIR and Raman
spectra for GOSH taken before and after were confirmed by

Results conclusively show typical bands for GOSH that are preserved upon catalyst recycling: $C=O$ stretching (1640 cm⁻¹), preserved upon catalyst recycling: C=O stretching (1640 cm^{-1}) , N-H bending (3292 cm^{-1}) , N-H stretching (1542 cm^{-1}) , C-N stretching (1427 cm^{-1}) and N-H wagging (686 cm^{-1}) . Other observed bands are attributed to GO domains.⁷ The weak band Results conclusively show typical bands for GOSH that are

after recycling GOSH, which is attributed to the predominance after recycling GOSH, which is attributed to the predominance of deprotonated thiolate (reaction $pH > pK_a$). Even so, the amide bond preservation confirms the thiol group on GOSH. due to S-H stretching (2569 cm^{-1}) is hardly distinguishable

TGA analysis (Supplementary Information) confirms the characteristic mass losses of GOSH before and after recycling: (i) 120-230 $^{\circ}$ C, with \sim 15% mass loss due to oxygenated groups; (ii) 250-400°C, with \sim 25% mass loss attributed to thiol groups attached to GO domains and (iii) 400
loss due to GO carbon backbone. I
maintain intact after reaction, reiter loss due to GO carbon backbone. Hence, the thiol moieties maintain intact after reaction recycling is legitimate. amide bond preservation confirms the thiol group on GOSH.
TGA analysis (Supplementary Information) confirms the
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recycling is legitimate. oone. Hence, the thiol moieties
, reiterating that the proposed % mass loss due to oxygenated groups; 5% mass loss attributed to thiol groups
s and (iii) 400-550°C, with ~50% mass

Lastly, Raman spectra were obtained mainly to confirm GO-like nature. Indeed, results show (Supplementary Information) intense bands, characteristic for this kind of carbonaceous materials: D (1350 cm⁻¹), G (1580 cm⁻¹) and D^{\prime} (1607 cm^{-1}) bands.⁷ After recycling, these bands are maintained and the intensity ratio between D and G bands (I_D/I_G) , associated to the defective nature of the material (functionalized) is retained \sim 2.2, and no further major change (functionalized) is retained \sim 2.2, and no further major change occurs on GO surface. Also, the band at 2920 cm⁻¹ related to the CH ² asymmetric stretch of the thiol group was detected the CH₂ asymmetric stretch of the thiol group was detected after reaction.⁷ Therefore, post-reaction characterizations after reaction.⁷ Therefore, post-reaction characterizations confirm the recycling features of GOSH, with no indication of thiol leaching, which is expected since it is covalently attached to the GO surface by highly stable amide bonds. Lastly, Raman spectra were obtained mainly to confirm
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In conclusion, the present study reports for t

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the disulphide of deproted by during the amide book recovered by characterized by characterized by converted by contracted in the main main intuition in the main ma In conclusion, the present study reports for the first time the use of GO-based materials with impressive catalytic activity in dephosphorylation reactions. Additionally, the nanocatalyst comprising reactive thiol groups, GOSH, can effectively be recycled consecutively, without losing its catalytic activity significantly. All characterization carried out confirmed that the catalyst maintained its overall functionalities after reuse. A mechanism involving nucleophilic attack by thiolate was proposed, mimicking enzymatic reactions involving thiol-based groups. Therefore, the nanocatalyst proposed has certainly groups. Therefore, the nanocatalyst proposed has certainly
innovative features, particularly promising in designing
artificial enzymes, by exploring its multifunctionalities, e.g.,
coupling GOSH with therapeutic agents, en artificial enzymes, by exploring its multifunctionalities, e.g. coupling GOSH with therapeutic agents, envisioning advances in genetic therapy. In this sense, we believe that targeted functionalization of GO and overall engineering of carbon nanomaterials broadens the field of novel complex multifunctional catalyst which can combine assembly characteristics and multiple catalyses (nucleophilic, acid-base). It is noteworthy that these catalysts are also very promising in the detoxification of chemical warfare and pesticides from the It is noteworthy that these catalysts are also very promising in the detoxification of chemical warfare and pesticides from the phosphate ester family.²¹ There is a great interest in developing phosphate ester family.²¹ There is a great interest in developing new and efficient methods to detoxify these agents to eliminate new and efficient methods to detoxify these agents to eliminate stocks, treat population and contain attacks. These concerns are stocks, treat population and contain attacks. These concerns are
evident by the 2013 Peace Nobel Prize awarded to the Organisation for the Prohibition of Chemical Weapons for its efforts in eliminating chemical weapons. GOSH can be readily used for detoxification purposes, since it effectively cleaved DEDNPP, a substrate similar to known toxic agents (e.g. paraoxon). This attribution is not unprecedented since it is common to evaluate detoxification dephosphorylation reactions. Additionally, the nanocatalyst comprising reactive thiol groups, GOSH, can effectively be recycled consecutively, without losing its catalytic activity significantly. All characterization carri catalyst maintained its overall functionalities after reuse. A mechanism involving nucleophilic attack by thiolate was proposed, mimicking enzymatic reactions involving thiol-based groups. Therefore, the nanocatalyst propo innovative features, particularly promising in designing
artificial enzymes, by exploring its multifunctionalities, $e.g.,$
coupling GOSH with therapeutic agents, envisioning advances
in genetic therapy. In this sense, we b evident by the 2013 Peace Nobel Prize awarded
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efforts in eliminating chemical weapons.²² The nano-
GOSH can be readily used for detoxification purposes,
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It is noteworthy that these catalysts are also very promising in
the detoxification of chemical warfare and pesticides from the
phosphate ester family.²¹ the of Chemical Weapons for its in eliminating chemical weapons.²² The nanocatalyst can be readily used for detoxification purposes, since it ely cleaved DEDNPP, a substrate similar to known In and contain attacks. These concerns are
3 Peace Nobel Prize awarded to the
Prohibition of Chemical Weapons for its
1, chemical weapons.²² The nanocatalyst GOSH can be readily used for detoxification purposes, since it effectively cleaved DEDNPP, a substrate similar to known toxic agents (*e.g.* paraoxon). This attribution is not unprecedented since it is common to evaluate d study reports for the first time the
th impressive catalytic activity in
Additionally, the nanocatalyst
vups, GOSH, can effectively be
out losing its catalytic activity
ion carried out confirmed that the
lll functionalitie based
tainly
gning
e.g., properties in model substrates. Finally, studies that involve dephosphorylation processes by a clear-cut catalysis are promising for both development of artificial enzymes and detoxification of organophosphorus agents.

 Authors acknowledge the financial support from CNPq, CAPES, Fundação Araucária, NENNAM (PRONEX Fundação Araucária/CNPq) and National Institute of Science and Technology of Carbon Nanomaterials (INCT-Nanocarbono).

Notes and references

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† Electronic Supplementary Information (ESI) available: Potentiometric titration data, kinetic profiles, TGA curves and Raman spectra. See DOI: 10.1039/c000000x/

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